

Please substitute the following paragraph on page 6, beginning at line 14:

According to a further aspect of the present invention, generally a method for the in vitro or in vivo degradation of amorphous or crystalline silicone dioxide (condensation products of the silicic acid, silicates), silicones and other silicon (IV)- or metal (IV)-compounds as well as of mixed polymers of these compounds is provided, wherein a polypeptide or a metal complex of a polypeptide is used for the degradation, characterized in that the polypeptide comprises an animal, bacterial, plant or fungal carbonic anhydrase domain that exhibits a sequence similarity of at least 25% ~~(see Figure 3)~~ (see Figures 3A and 3B) to the sequence shown in SEQ ID No. 1. Until now, it was not known that such carbonic anhydrase-domains- containing enzymes are able to decompose such silicates or silicones. Due to the reversibility of the process a further aspect of the present invention relates to a method for the synthesis of amorphous silicone dioxide (condensation products of the silicic acid, silicates), silicones and other silicon (IV)- or metal (IV)-compounds as well as of mixed polymers of these compounds, wherein a polypeptide or a metal complex of a polypeptide is used for the synthesis, characterized in that the polypeptide comprises an animal, bacterial, plant or fungal carbonic anhydrase domain that exhibits a sequence similarity of at least 25% to the sequence shown in SEQ ID No. 1.

Please substitute the following paragraph on page 10, line 25:

~~Figure 2 (below)~~ Figure 2B shows the nucleotide sequence of the sponge-silicase-cDNA - identified with the aid of the differential display technique -, and ~~Figure 2 (above and below)~~ Figures 2A and 2B, as well as Figure 3A show the polypeptide derived from the nucleotide sequence of the sponge-silicase (SIA_SUBDO).

Please substitute the following paragraph on page 22, line 15:

These enzymes catalyse the reversible hydration of carbon dioxide ~~(Figure 8 [1])~~ (Figure 8A). Carbon dioxide is converted into HCO_3^- and H^+ by the carbonic anhydrase.

Please substitute the following paragraph on page 22, line 18:

The silicase indeed also exhibits a carbonic anhydrase-activity, as could be shown with a colorimetric assay (Armstrong JM, Myers DV, Verpoorte JA, Edsall JT (1966) Purification and

properties of human erythrocyte carbonic anhydrase. J Biol Chem 241: 5137-5149). Accordingly, it is possible that the silicase causes a change of the pH because of the conversion of CO_2 into HCO_3^- (~~Figure 8 [1])~~ (Figure 8A). This allows for an etching of lime substrates, but not of silicon dioxide-materials, whose solubility increases with increasing but not lowering pH.

Please substitute the following paragraph on page 22, line through page 23, line 8:

It is known that three histidine residues are involved in the mode of action (carbonic anhydrase-activity) of the carbonic anhydrase that bind to a divalent zinc ion; accordingly, the following mode of action can be formulated for the silicase-activity (~~Figure 8 [2])~~ (Figure 8B). In the silicase of *S. domuncula* the histidine residues are found in the derived polypeptide at the amino acid positions aa₁₈₁, aa₁₈₃ and aa₂₀₆ (Figure 3A). In water (Lewis-base) a hydroxide anion is formed that is bound to the Zn^{2+} (Lewis-acid). This performs a nucleophilic attack at one of the silicon atoms that are linked one with the other by oxygen atoms (~~Figure 8)~~ (Figure 8B). In the next step the zinc-complex binds to the silicon atom by cleaving of the oxygen bond. Under consumption of H_2O finally a free silicic acid is released the initial zinc(II)-bound hydroxide anion is formed again.

Please substitute the following paragraph on page 27, line 20:

Figure 2: top: Figures 2A and 2B:

Figure 2A: Amino acid sequence derived from the nucleotide sequence of the open reading frame (coding region) of the *S. domuncula* silicase-cDNA (SIA_SUBDO). ~~bottom: Figure 2B:~~ Nucleotide sequence of the *S. domuncula* Silicase-cDNA (SIA_SUBDO). The amino acid sequence derived from the nucleotide sequence of the open reading frame is given below the nucleotide sequence.

Please substitute the following paragraph on page 27, line 27:

Figure 3:(A) Figures 3A and 3B:

Figure 3A: Alignment of the *S. domuncula* silicase (SIA_SUBDO) with the human carbonic anhydrase 11 (carbonate dehydratase II) (CAH2_HUMAN; P00918). The carbonic anhydrase domain is framed (|= e-CA_{dom} =|). The characteristic amino acids that form the eukaryotic-type-carbonic anhydrase-signature, are marked (▲: found in both sequences; ▫: present only in the carbonic anhydrase but not in the silicase). The additional symbols (+) indicate those residues, that form the

hydrogen-network of the active center. The three zinc-binding histidine-residues are marked (Z). Similar amino acid residues between both sequences are highlighted. The borders of the long (~rec~ to ~rec~) as well as the short recombinant silicase (~rec-s~ to ~rec~) are marked and underlined twice. ~~(B)~~ **Figure 3B:** phylogenetic tree, constructed with the sponge-silicase and following related enzymes: human carbonanhydrase I (carbonate dehydratase I) (CAH1_HUMAN; P00915), II (CAH2_HUMAN), III (CAH3_HUMAN; P07451), IV (CAH4_HUMAN; P22748), VI (CAH6_HUMAN; P23280), VII (CAH7_HUMAN; P43166), VIII (CAH8_HUMAN; P35219), IX (CAH9_HUMAN; Q16790), X (CAHA_HUMAN; Q9NS85), VA (CAH5_HUMAN; P35218), VB (CA5B_HUMAN; Q9Y2D0), XII (CAHC_HUMAN; 043570), XIV (CAHE_HUMAN; Q9ULX7), carbonic anhydrase of *Caenorhabditis elegans* (CAH_CAEEL; Nu-510674.1), carbonic anhydrase of *Drosophila melanogaster* (CAH1_DROME; NP523561.1), carbonic anhydrase of the plants *Arabidopsis thaliana* (CAH-I_ARATH; NP_196038.1) and *Chlamydomonas reinhardtii* (carbonate dehydratase 1) (CAH1CHLRE ; P20507) as well as bacterial carbonic anhydrases from *Neisseria gonorrhoeae* (CAH_NEIGO; Q50940), *Klebsiella pneumoniae* (CAH_KLEPN; 052535) and the cyanobacteria *Nostoc* sp. PCC 7120 (CAHANASP; P94170). The latter sequence were used as outgroup. The measure bars indicate an evolutionary distance of 0,1 amino acid-substitutions per position in the sequence. The phylogenetic tree was constructed by means of "Neighbor-Joining" ("Neighbor" program: Felsenstein, J. (1993). PHYLIP, ver. 3.5. University of Washington, Seattle).

Please substitute the following paragraph on page 29, line 5:

Figure 6: Figures 6A-6D

Effect von silicon on the formation of spicules in Primmorphs. For the formation of the Primmorphs dissociated cells of the marine sponge *S. domuncula* were incubated in sea water, supplemented with 10% RPM 11640-Medium and 30 μ M Fe(+++). The Primmorphs were then transferred for 3 days into a medium (RPMI 1640, Fe(+++)) that was enriched with 60 μ M silicon. (A) The Primmorphs were incubated in medium plus silicon. magnification: x6. (B) In some cases the Primmorphs started with the synthesis of spicules (sp). magnification: x10. For the semi-quantitative determination, the Primmorphs were pressed between two cover slides (C and D). (C) Primmorphs that were incubated in the absence of silicon inkubierte were nearly completely without

spicules, whereas those that were cultivated in the presence of silicon contained newly formed spicules (>); (D); magnification: x200[.]

Please substitute the following paragraph at page 29, line 25:

Figure 8: Figures 8A and 8B:

Enzymatic reactions as mediates by the silicase (carbonic anhydrase) of *S. domuncula*. In Figure 8A [1] the conversion of CO_2 into HCO_3^- is shown. In [2] Figure 8B, the reaction of the silicase is shown. The silicase binds one zinc atom with its three histidine-residues. The zinc ion, a Lewis-acid, binds a hydroxide-anion that is derived from water, a Lewis-base. The silicase/zinc-complex undertakes a nucleophilic attack auf a silicon atom between the oxygen bonds. Thereby, the hydrolysis of the polymeric silicon dioxide is achieved, which first - with one of both product halves – maintains bound to the enzyme. Upon further consumption of H_2O , the product is released until finally free silicic acid is left after several cycles.

Please substitute the following paragraph at page 30, line 1:

Figure 9: Figures 9A and 9B:

~~Left-~~ Figure 9A: Spicules (needles) of *Suberites domuncula* after 6 hour incubation in the absence of silicase. ~~Right-~~ Figure 9B: Spicules of *Suberites domuncula* after 6 hour incubation in the presence of silicase. The incubation took place under the conditions as described in table 2.